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**Supporting information for article:**

**The deduced role of a chitinase containing two nonsynergistic catalytic domains**

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**Table S1** The strategy and primers for the cloning of the gene encoding *OfChtIII*.

PCR fragment	Size (bp)	Primer	Primer sequence (5' - 3')
<i>OfCHTIII-a</i>	194	<i>O</i> fChtIII-F1	TCAGCTAGCGTGAACAGACCAAA
		<i>O</i> fChtIII-R1	TCAAAGTTCCGTGCCCTCAAGTA
		<i>O</i> fChtIII-F2	ATTCAGCCGATCTCTGCACCCA
		<i>O</i> fChtIII-R2	AACTTCTGAGTCCCAGAAAGACCA
<i>OfCHTIII-b</i>	760	3' RACE outer primer	TACCGTCGTTCCACTAGTGATT
		<i>O</i> fChtIII-F3	TGCACCCACATCATCTTCGCCTTC
		3' RACE inner primer	CGCGGATCCTCCACTAGTGATTCACTATAAGG
		<i>O</i> fChtIII-F4	ATGAGACCAAGGATGGCAAGACCG
<i>OfCHTIII-c</i>	2759	3' RACE outer primer	TACCGTCGTTCCACTAGTGATT
		<i>O</i> fChtIII-F5	AGGTGTGTGAGATTCTCGCAACG
		3' RACE inner primer	CGCGGATCCTCCACTAGTGATTCACTATAAGG
		<i>O</i> fChtIII-F6	GACGACGAAATGAAGGTGCCGTAC
<i>OfCHTIII-d</i>	772	5' RACE outer primer	GCTGATGGCGATGAATGAACACTG
		<i>O</i> fChtIII-R3	GGTAGGGGATGGCTGAGTAGATGA
		5' RACE inner primer	CGCGGATCCGAATTAATACGACTCACTATAAGG
		<i>O</i> fChtIII-R4	GGGTCGCTGACATCTCCTTGAAC

**Table S2** Sequences of primers for cloning of *OfChtIII* and its mutants and truncations.

	Primer	Primer sequence (5' - 3')
<i>OfChtIII</i>	<i>OfChtIII</i> -F	GAGAGGCTGAAGCTTACGTAGAATT CGTCTCCGTACATCCTCGGT GTCC
	<i>OfChtIII</i> -R	AATT CGCGGCCGCTAACATGATGATGATGATGAGCGGCCGGTGCT TGC
CAD2	CAD2-F	AGCTTACGTAGAATT CGAGCCCCAAGTCCTCTGCTAC
	CAD2-R	AATTAATT CGCGGCCGCTAACATGATGATGATGATGATGTGACTCGTA AGGTCCCGTCG
CAD1-E217L	E217L-F	TGGACATCGATTGGTTGTACCTAACCTAACGGCGGAGAC
	E217L-R	CCTTAGGGTACAACCAATCGATGTCCAGACCCTCG
CAD2-E647L	E647L-F	TGGACGTCGACTGGTTGTACCCAAGAGGGAGCAGAT
	E647L-R	CTCTTGGGTACAACCAGTCGACGTCCAGACCCTTG

**Table S3** Sequences of primers for quantitative RT-PCR.

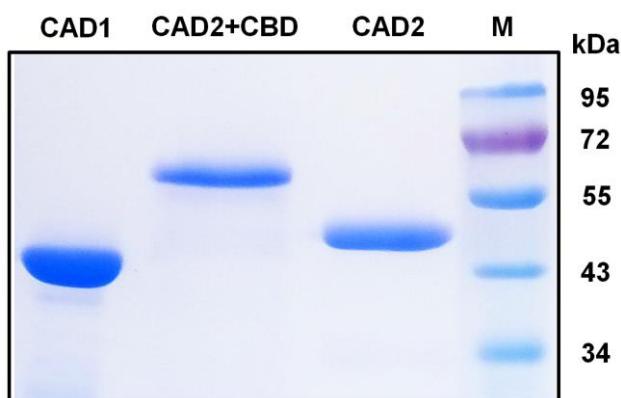
Gene name	Primer name	Primer sequence (5' - 3')
<i>OfCHTI</i>	<i>OfCHTI</i> -F	GGCGACCCTATT CCTACCACGAC
	<i>OfCHTI</i> -R	GCGCCTCTCCTCCCGTCGTC
<i>OfCHTIII</i>	<i>OfCHTIII</i> -F	AGCCCGAACTCTGCACCC
	<i>OfCHTIII</i> -R	AACCCAGTCTGCCATCCTTG
<i>OfCHSA</i>	<i>OfCHSA</i> -F	ACGGATTGGATGATGATTACGAC
	<i>OfCHSA</i> -R	CGTCAAAGTGCCAATGTTCC
<i>RPS3</i>	<i>OfRPS3</i> -F	TGCAACGACTACGTCAACACC
	<i>OfRPS3</i> -R	TCGGGCTGCGGTTCTT

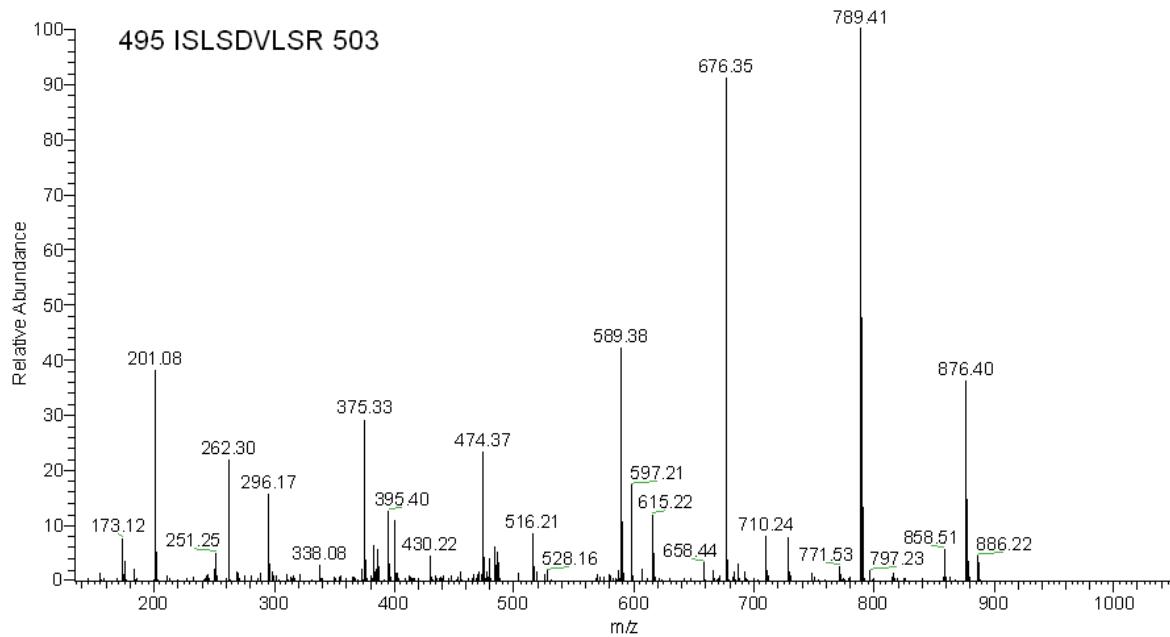
**Table S4** Kinetic parameters of CAD1 and CAD2 toward ethyl glycol chitin.

	$K_m$ (mg ml $^{-1}$ )	$k_{cat}$ (s $^{-1}$ )	$k_{cat}/K_m$ (s $^{-1}$ mg $^{-1}$ ml)
CAD1	1.10±0.09	3.02±0.19	2.75
CAD2	2.19±0.17	6.06±0.11	2.77

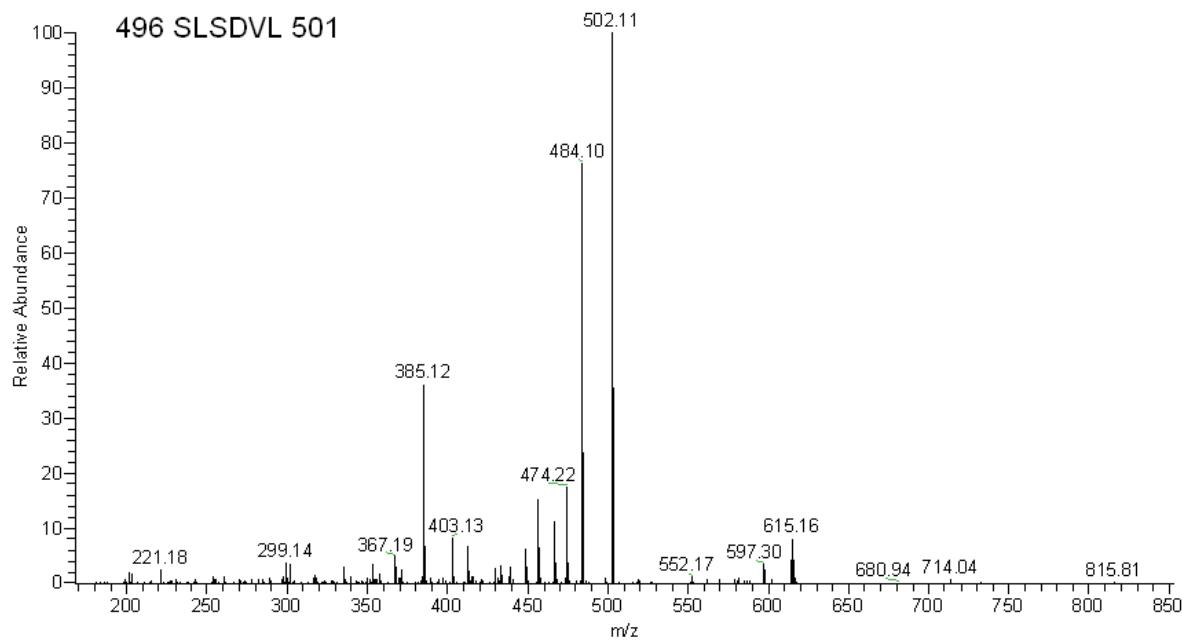
**Table S5.** The results of Cremer-Pople parameter calculation of -1 GlcNAcs.

	$\phi$ (°)	$\theta$ (°)	
Pyranose in ideal $^1S_5$ conformation	270	90	
-1 GlcNAc of (GlcNAc) <sub>6</sub> in GH18A-E217L	262	90	
-1 GlcNAc of (GlcNAc) <sub>5</sub> in GH18B-E647L	257	88	

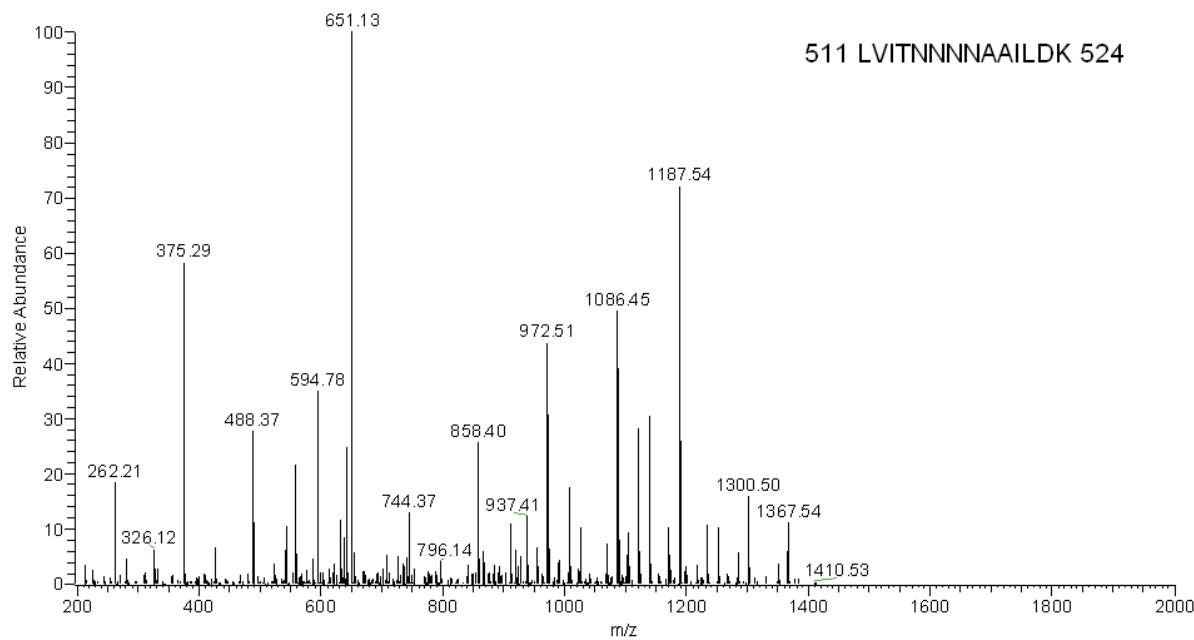
**Figure S1** SDS-PAGE analysis of the purified CAD1, CAD2-CBD and CAD2.



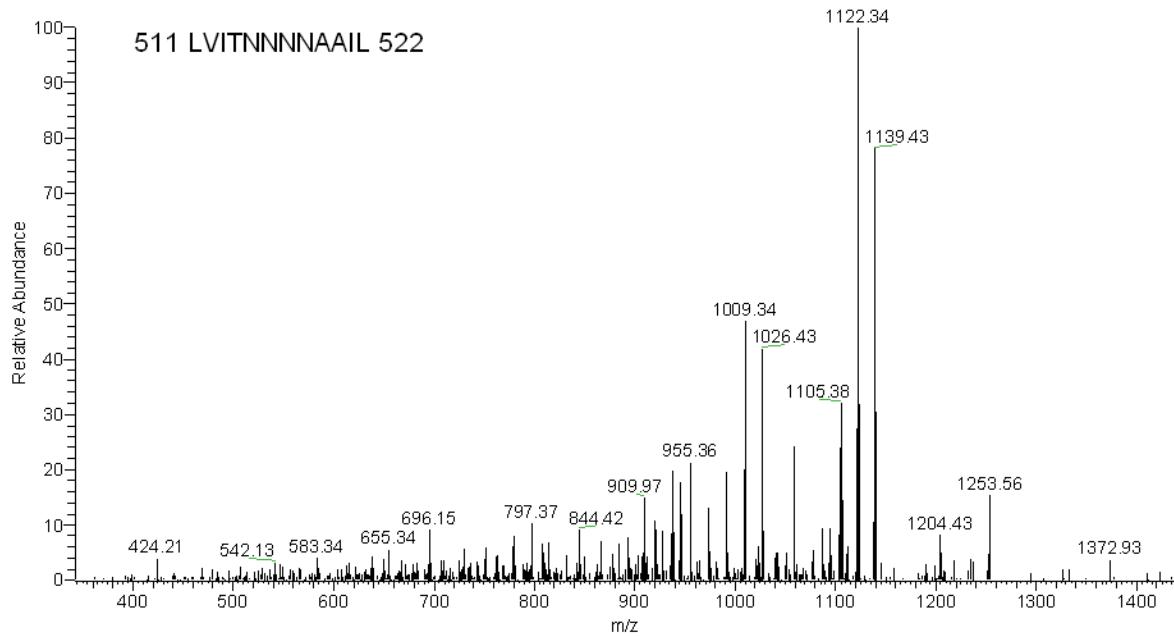
**Figure S2** MS/MS spectrum of C-terminal peptide of CAD1 from trypsin digestion.



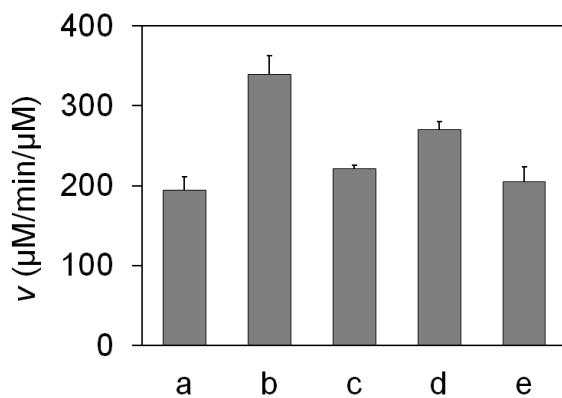
**Figure S3** MS/MS spectrum of C-terminal peptide of CAD1 from chymotrypsin digestion.



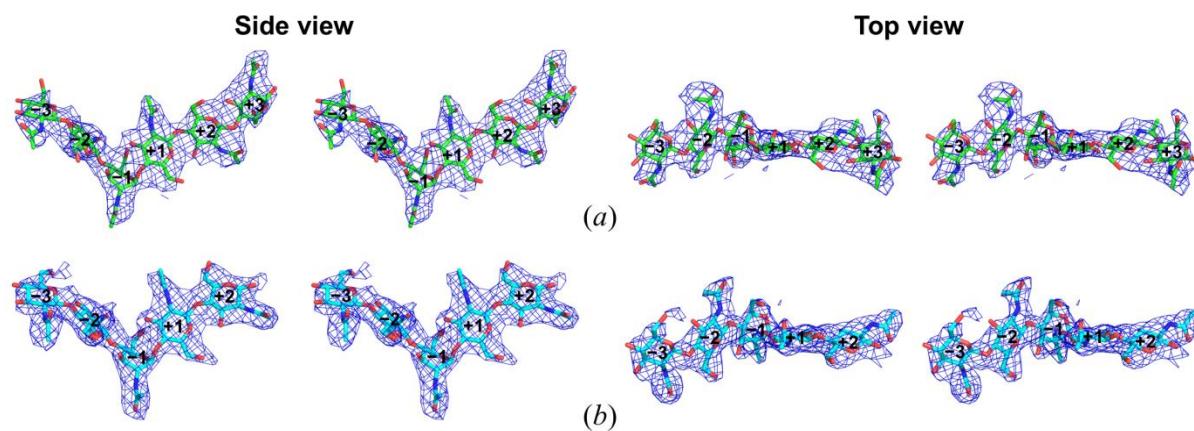
**Figure S4** MS/MS spectrum of N-terminal peptide of CAD2 from trypsin digestion.



**Figure S5** MS/MS spectrum of N-terminal peptide of CAD2 from chymotrypsin digestion.



**Figure S6** Synergism of GH18A, GH18B and GH18B-CBM14 on the degradation of ethylene glycol chitin. a, GH18A; b, GH18B; c, GH18B-CBM14; d, GH18A+GH18B; e, GH18A, GH18B-CBM14.



**Figure S7** The stereo views of the simulated-annealing composite omit maps of  $(\text{GlcNAc})_6$  in GH18A-E217L (a) and  $(\text{GlcNAc})_5$  in GH18B-E647L (b). The composite omit map was calculated using *PHENIX* program with simulated-annealing at 3,000 K. The  $2F_o - F_c$  map around the ligand is contoured at the  $1.0 \sigma$  level.